J. Appl. Biol. Sci., 19 (1): 1-7, January 2025
DOI: https://doi.org/10.71336/jabs.1371
RESEARCH ARTICLE

Journal of Applied Biological Sciences
E-ISSN: 2146-0108; License: CC BY 4.0
Anatolia Academy of Sciences Open Access

Comparative antibacterial activity of silver nanoparticles from *Blumia lacera* and *Neolamarckia cadamba* against methicillin-resistant *Staphylococcus aureus*

Kalpesh Khutade[1]*, Harshila Dhinde[2], Nisha Kumari[3], and Harshada Shah[4]

Faculty of Microbiology, Vedantaa Institute of Medical Sciences and Research Center, Dahanu-401606, Maharashtra, India

*Correspondence:
Mr. Kalpesh Khutade
Email: kalpeshkhutade111@gmail.com

Received: June 27, 2024
Revised: January 20, 2025
Accepted: January 21, 2025

ABSTRACT: Silver nanoparticles (AgNPs) have shown promise as antibiotic-free antibacterial agents. This study comparatively evaluates the antibacterial activity of AgNPs synthesized from *Blumia lacera* and *Neolamarckia cadamba* against methicillin-resistant *Staphylococcus aureus* (MRSA). *Staphylococcus aureus* isolates obtained from various clinical samples, including wound swabs and pus, were phenotypically and genotypically confirmed as MRSA. AgNPs were characterized using UV-visible spectroscopy, and their antibacterial efficacy was assessed via disc diffusion, agar cup methods, and time-kill curves. Results demonstrated that *Blumia lacera* AgNPs exhibited remarkably significant ($P < 0.009$) antibacterial activity by showing greater zones of inhibition compared to *Neolamarckia cadamba*. These findings highlight the potential of green-synthesized AgNPs as effective agents against MRSA.

KEYWORDS: AgNPs, Ultraviolet visible spectroscopy, Methicillin-resistant *Staphylococcus aureus* (MRSA), Green synthesis, Antimicrobial activity

INTRODUCTION

Infections with bacteria can impede the healing of wounds, particularly in those with severe burns or long-term medical conditions. The regular antibiotic medication that was initially effective has become less effective as drug-resistant bacteria emerge [1]. A significant cause of *Staphylococcus aureus* (S. aureus) is gram-positive organisms, which have the highest isolation rate [2].

It can lead to infections in various body parts in clinical specimens, such as urine, sputum, wound pus, blood, and fluids from the abdomen and chest. Methicillin-resistant *Staphylococcus aureus* (MRSA) is more common due to the overabundance of antibiotics. This has increased healthcare costs, as well as mortality and morbidity from MRSA [3]. There is an urgent need for new treatment alternatives, especially those based on novel antimicrobial drugs that are less prone to acquiring resistance and can treat infections through new mechanisms [4, 5]. Apart from the investigation and creation of novel organic antibiotics, inorganic antibacterial compounds could serve as appropriate substitutes. Therefore, it is possible to use silver nanoparticles (AgNPs) as A promising weapon against MRSA [6].

Recenly, the promising potential antibacterial agents for treating infected wounds, such as carbon-based nanomaterials, nanocomposites, silver nanoparticles (AgNPs), and metal oxides [7]. AgNPs have good stability, broad spectral action, and minimum cytotoxicity [8]. AgNPs have to prevent the growth of bacterial biofilm because they have excellent antibacterial properties [9]. The various biological actions of AgNP include improving the immunogenicity of vaccines, promoting wound repair, anticancer activity, and having antidiabetic effects. In the early nineteen century, for wound infection, AgNPs were commonly used in wound treatment [10]. By controlling the synthesis of inflammatory substances or proteins, AgNPs can help accelerate the healing of wounds [11]. The importance of AgNPs in medicine has increased significantly with the use of antibiotics in curing infected wounds decreases [12]. Silver is a heavy metal that is harmful if it builds up in the body, but when used properly, it can still be beneficial for wound healing [13]. Moreover, AgNP-containing nanocomposites, featuring gels and dressings, can also significantly lessen the harm that AgNPs do to human beings and reduce the possibility of their spreading to wounds [14].

How to cite this article: Khutade K, Dhinde H, Kumari N, and Shah H. (2025). Comparative antibacterial activity of silver nanoparticles from *Blumia lacera* and *Neolamarckia cadamba* against methicillin-resistant *Staphylococcus aureus*. *Journal of Applied Biological Sciences*, 19 (1): 1-7. https://doi.org/10.71336/jabs.1371

The Ag+ having greater toxicity to HepG2 cells. However, there is no unified statement on their therapeutic effects, making it important to compare their effects on infected wounds. This study aims to compare the antibacterial efficacy of *Blumia lacera* and *Neolamarckia cadamba* derived silver nanoparticles against MRSA, with a focus on their potential application in wound healing.

MATERIALS AND METHODS

Collection of clinical wound samples

Samples (wound swabs and pus) were collected among the patients in the Surgery Department at the Vedantaa Institute of Medical Sciences, Vedantaa Hospital and Research Centre, Dhundalwadi, Maharashtra State, using the swab technique. The identification and detection of MRSA using phenotypic and molecular methods.

Identification of methicillin-resistant *S. aureus*

Staphylococcus aureus was isolated and identified through culture, Gram staining, and biochemical tests on Nutrient agar and Mannitol salt agar**.** A cefoxitin (30 g) disc was used in place of methicillin. An inhibition zone diameter of 19 mm indicated resistance, but an inhibition zone diameter of 20 mm indicated sensitivity to methicillin [15]. MRSA was cultured at 37 °C throughout the entire night in nutrient broth (NB). After being cleaned, the cells were suspended in NB, and the optical density (OD) was set to 0.1, or 10^8 CFU/ml at a wavelength of 600 nm.

DNA extraction

Colonies of cefoxitin-resistant *Staphylococcus aureus* were cultured in nutrient broth for 24 hours at 37 °C, with occasional shaking and a 5-minute microcentrifuge spin at the maximum speed of 1000 rpm. After that, the cell suspension was pipetted and centrifuged at 4000 rpm for 10 min in a separate test tube. Total genomic DNA was isolated from using boiling lysis method by re-suspending at cell pellet of1 g in 50UL water which were then boiled for 15 minutes at a temperature of102 to110 degrees Celsius. The cooling was performed on ice to the sample place and for 1 min to 5000 rpm it wa centrifuged. The supernatant was resuspended in cold 95% ethanol and kept at -20 °C until PCR was performed [16].

Polymerase chain reaction (PCR) assay

PCR mix contained 50 ul of distilled water, Buffer 1x, MgCl2, Taq polymerase fields, dNTP pump, MecA primer (Table 1) and was prepared in a 0.2-ml tube. A PCR mixture was prepared in 20ul volume and 10ul DNA template was added to the PCR mixture and placed inside the Insta Q96 (Himedia) thermal cycler for the amplification process This process comprised initial denaturation at 92 °C for 30 minutes, denaturation at 92 °C for 10 minutes, annealing at 56 °C for 10 min and extension at 72^0C for another 10 min.

Table 1. Oligonucleotide sequence of PCR primers employed for the identification of mecA gene.

Name of primers	Sequence 5'-3'
MecA-F	GTAGAAATGACTGAACGTCCGATTA
MecA-R	CGAATTCGACATTGTTTCCGTCTAA

* Source: [17]

Synthesis of nanoscale silver particles by the wet reduction method

To make the plant leaf broth solution, 5 g of cleaned chopped leaves were blended in a 500-ml flask with 100 ml of sterile D/W and boil 15 minutes. After being refrigerated for a week, the broth was used. One milliliter of silver nitrate solution (AgNO3) and an equal amount of prepared leaf extract were mixed well in a beaker. The beaker was heated for 3 hours at 30 °C. After 3 hours, the solutions were removed and cooled. Ag-NPS were formed.

CFU assays and time-kill measurements

In 100 milliliters of NB supplemented with several concentrations of nanosilver (total silver content: 5, 20, 50, or 100 µg) at 37 °C under constant agitation, MRSA cells were cultivated in order to determine the killing kinetics in the presence of Ag-NPS. The cylindrical sample containers were held horizontally with an orbital shaker while 300 rpm of agitation was administered. The optical density- MRSA cells (CFU/ml) (OD) at 600 nm was used to assess the amounts of bacterial concentrations.The viable count by the spread plate method using nutrient agar media was used to observe the number of MRSA cells. The study confirmed a decrease in the viable count and the percentage of surviving MRSA in the *Blumia lacera* and *Neolamarckia cadamba* nanosilver groups after incubation for various durations (0 hrs, 1 hrs, 2 hrs, and 3 hrs).

Disk diffusion method

In this method, 0.1 ml of a selected strain of bacterial culture at 0.1 O.D. was swabbed on a sterile Mueller-Hinton agar plate. Whatman No. 1 filter paper discs were dipped in nanosilver solution (0.001 M), placed on an agar plate, and incubated for 24 hours at 37 °C. Inhibition zones were measured in mm [18].

Agar cup method

Fifteen milliliters of molten Mueller-Hinton agar media was bulk seeded with 1 ml of the bacterial culture and aseptically poured onto a sterile plate. The plates were allowed to set. The cup was made into a plate with the help of a cork borer, and 0.1 ml of nanosilver solution (0.001 M) was put to the well. Zones of inhibition (mm) were observed after 48 hours and the results are recorded. The results obtained for the test organism were then compared for Ag-*Blumia lacera* and Ag-Neolamarckia cadamba.

Statistically analysis

In this study, a paired t-test was conducted (https://www.statskingdom.com/paired-t-test-calculator.html) to analyze the differences in microbial inhibition zones between the disc diffusion method and agar cup method across various concentrations (undiluted, 1:10, 1:25, and 1:50) of antimicrobial agents. This statistical test was used to assess the significance of the differences in inhibition zone diameters for two different methods of antimicrobial testing. The chi-square test was also applied to examine the data, with a p-value of less than 0.05 indicating a significant difference.

RESULTS AND DISCUSSION

Characterization of Ag-NPS by UV Spectroscopy

Ag-NPS was obtained from *Blumia lacera* and *Neolamarckia cadamba* leaf extracts via the wet reduction method. The surface plasmon resonance normally produces a strong peak between 400 and 420 nm in the case of Ag-*Blumia lacera* and 380–400 nm in Ag-*Neolamarckia cadamba*. Thus, Ag-NPs were characterized using a UV spectrophotometer (Figure 1). They found the antibacterial potential of green-synthesized AgNPs toward MRSA strains. Green synthesized silver nanoparticle: the color of solution changes to reddish-brown (formed in Milli-Q-water) and yellowish (formed in green tea extract). The peak intensity [400 nm (blue)] in the UV–Vis spectrum confirmed the synthesized silver nanoparticles were green. These results were in accordance with the UV–visible spectrum spectroscopy data as reported by Audtarat et al. [19].

Bacterial survival in the presence of Nanosilver

Methicillin-resistant *Staphylococcus aureus* survival of *Blumia lacera* and *Neolamarckia cadamba* synthesized nanosilver particles was investigated through two methodologies. Bacterial survival of the *Blumia lacera* and *Neolamarckia cadamba* nanosilver particles was determined by detecting absorbance using spectrophotometer at 550 nm (Figure 2). In all undiluted solutions of *Blumia lacera* Ag-NPs, maximum antibacterial activity and highest survival rate of MRSA was found as follows. This was further confirmed by viable count. The percentage of MRSA surviving was further decreased in case of *Blumia lacera* and *Neolamarckia cadamba* nanosilver particles when incubated with particles for 0 hrs, 1 hrs, 2 hrs and 3 hrs. The cell number of the undiluted *Neolamarckia cadamba* Ag-NPs had a zero viable cell count after 2 hours of incubation suggesting this agent was less effective at higher concentration than both the surface and nut blight nanoparticles (Table 2).

The plate diffusion and agar well test further confirmed this result. In the present study, we observed that *Neolamarckia cadamba* nanosilver had a relatively lower survival rate decreased post incubation and was not effective at higher concentrations compared to *Blumia lacera* nanoparticles. Similarity: Haque et al. [20] Time-kill curves on *E. coli* treated with AgNPs at different concentrations Results: The CFU counts were significantly reduced and up to 83% killing was observed in E.coli by 40 mg/mL AgNPs. At 80 and 10 mg/mL, AgNPs were bactericidal with 88% and 28% killing of *E. coli* respectively. The time-kill kinetics of, CS-AgNPs against different human pathogens were assessed to determine the bactericidal activity [21].

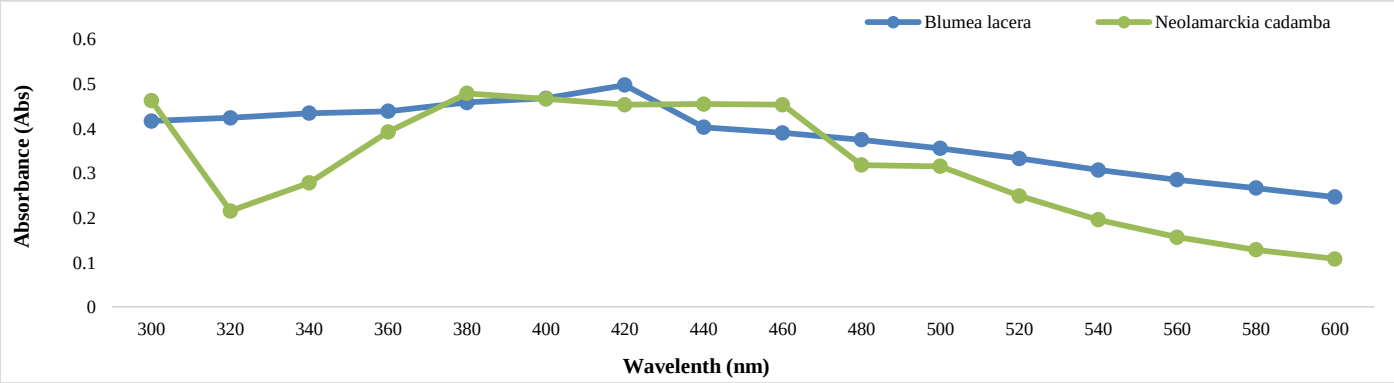

Figure 1. Green synthesis, surface plasmon resonance characterization, and antibacterial evaluation of silver nanoparticles from Blumea lacera and Neolamarckia cadamba leaf extracts against MRSA.

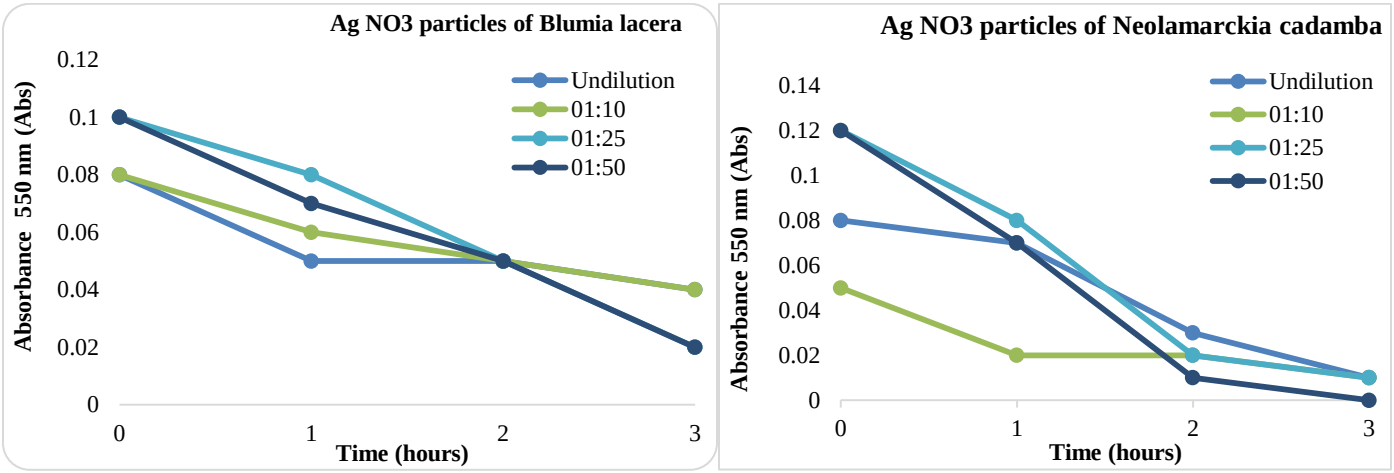

Figure 2. Evaluation of Methicillin-Resistant Staphylococcus aureus survival inhibition by Blumea lacera and Neolamarckia cadamba synthesized nanosilver particles using spectrophotometric Analysis.

Table 2. Time-dependent survival inhibition of Methicillin-Resistant Staphylococcus aureus by Blumea lacera and Neolamarckia cadamba nanosilver particles was evaluated, focusing on the effects of incubation duration and concentration on viable cell count

Dilution	**Undiluted $AgNO_3$**				**1:10 ml diluted $AgNO_3$**			
Time (hrs)	0	1	2	3	0	1	2	3
Blumea lacera (Average/cfu/ml)	1.30×10^8	2.26×10^7	5.20×10^6	4.10×10^6	1.46×10^8	1.25×10^8	1.17×10^7	1.31×10^7
Neolamarckia cadamba (Average/cfu/ml)	3.7×10^6	2.0×10^6	-	-	4.1×10^6	3.0×10^6	-	-
Dilution	**1:25 ml diluted $AgNO_3$**				**1:50 ml diluted $AgNO_3$**			
Time (hrs)	0	1	2	3	0	1	2	3
Blumea lacera (Average/cfu/ml)	1.71×10^8	1.44×10^8	1.17×10^8	2.34×10^7	2.72×10^8	1.72×10^7	1.89×10^7	3.0×10^6
Neolamarckia cadamba (Average/cfu/ml)	5.8×10^6	3.7×10^6	3.0×10^6	-	1.53×10^8	5.1×10^6	3.4×10^6	-

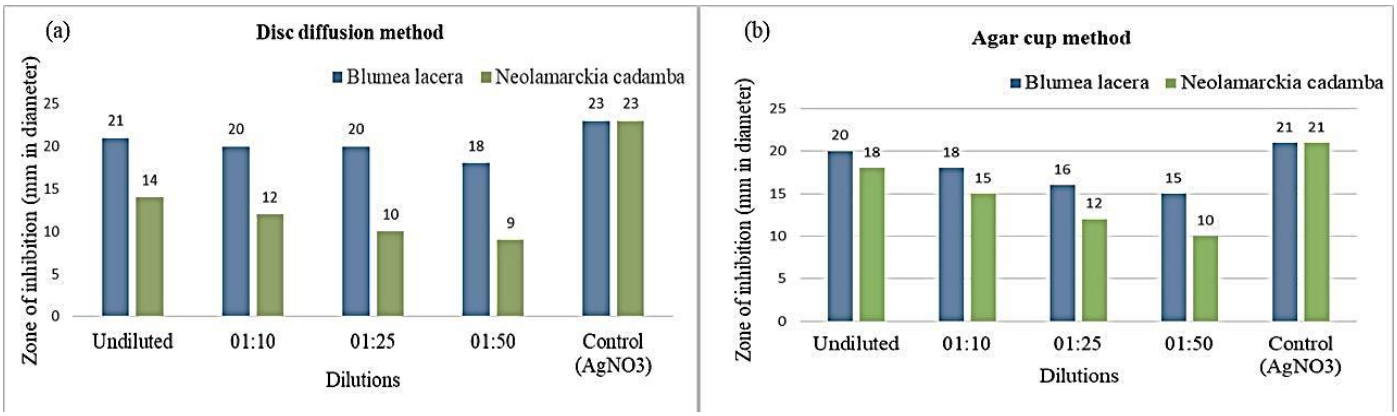

Figure 3. Antibacterial activity of silver nanoparticles derived from Blumea lacera and Neolamarckia cadamba against MRSA using disc diffusion and agar cup methods. Zones of inhibition (in mm) are shown for different concentrations (undiluted, 1:10, 1:25, 1:50) for both plant-derived AgNPs.

Antimicrobial activity

Ag-NPS activity against MRSA against undiluted, 1:50 ml, 1:25 ml and 1 - 10 ml was determined by using the disc diffusion method and agar cups. It is observed that the maximum zone of inhibition was in *Blumia lacera* compared to *Neolamarckia cadamba* nanosilver particles thus results are showed in (Figure 3).

The paired t-test results showed a statistically significant difference between the disc diffusion method (M = 20.4, SD = 1.8) and the agar cup method (M = 18, SD = 2.5), with a t-statistic of 4.7 (df = 4) and a p-value of 0.009. The disc diffusion method produced a larger average inhibition zone compared to the agar cup method. The t-statistic of 4.7 suggests a large difference relative to the variability. Thus, the difference between the two methods is both statistically significant and practically meaningful for *Blumia lacera*.

For the *Neolamarckia cadamba* silver nanoparticles, the paired t-test results showed a non-significant difference between the disc diffusion method (M = 13.6, SD = 5.6) and the agar cup method (M = 15.2, SD = 4.4), with a t-statistic of 1.6 (df = 4) and a p-value of 0.195. The t-statistic of 1.6 suggests a moderate difference, but the data does not provide strong evidence to reject the null hypothesis.

Broad-spectrum antibacterial activity was observed with the synthesized AgNPs on gram-positive bacteria [22]. The antibacterial activity of AgNPs against *S. aureus* was noticed with great importance by the agar well diffusion method [23]. Bioassay of *Penicillium* derived AgNP were found to have antimicrobial activity against the multi-drug resistant pathogenic species *S. aureus*, as per disc diffusion method [24]. When 80 µL of AgNP was used the maximum zones of inhibition were 21 mm and 20 mm, respectively. Likewise, similar effects on *S. aureus* were reported [25]. Consequently, ecofriendly green synthesized silver nanoparticles successfully exhibit antibacterial activity on various bacterial strains.

This is the first research article to compare the antibacterial properties of nanoparticles derived from two specific plant sources, which adds uniqueness and asserted its novel contribution to the field. Additionally, the study emphasizes the importance of identifying effective, antibiotic-free agents for combating drug-resistant bacteria.

The *Blumea lacera* AgNPs exhibit superior antibacterial properties when compared to *Neolamarckia cadamba* AgNPs. Studies have shown that smaller nanoparticles typically exhibit greater antibacterial activity due to their higher surface area-to-volume ratio [26]. This increased surface area allows for more active sites to interact with bacterial cells, potentially leading to more effective disruption of bacterial membranes and internal cellular processes [27]. In contrast, larger nanoparticles may have less surface area available for interaction, which could result in a reduced antimicrobial effect [28]. Thus, the small size of *Blumea lacera* AgNPs (12.52 nm) [29] may contribute to their superior ability to penetrate bacterial cell walls and inhibit bacterial growth more effectively than *Neolamarckia cadamba* AgNPs (80-200 nm) [30].

Another crucial factor is the surface charge of the nanoparticles. The charge of nanoparticles plays a significant role in determining their interaction with bacterial cell membranes, which are typically negatively charged. Positively charged nanoparticles tend to have a stronger attraction to the negatively charged bacterial membranes, allowing for better binding and, subsequently, more efficient penetration of the bacterial cell. This interaction can disrupt the integrity of the bacterial cell membrane, leading to leakage of intracellular contents and ultimately cell death [31]. The *Blumea lacera* AgNPs exhibit a more favorable surface charge for interaction with bacterial cells compared to *Neolamarckia cadamba* AgNPs; this may account for the former's enhanced antibacterial activity [32].

In addition to size and surface charge, the composition of the AgNPs can also significantly impact their antibacterial effectiveness. The synthesis methods used for fabricating these nanoparticles can introduce variations in their chemical composition, which may affect their toxicity and antimicrobial properties [33]. For instance, the presence of different stabilizing agents or capping agents could influence the stability and dispersibility of the nanoparticles, as well as their ability to release silver ions, which are responsible for their antimicrobial activity [34]. *Blumea lacera* AgNPs may have unique compositional features that enhance their antibacterial properties compared to *Neolamarckia cadamba* AgNPs, and these differences could be worth exploring in future studies.

CONCLUSION

In this study, the study revealed the presence of methicillin resistance strains confirmed by molecular identification. The antibacterial effect of AgNPs was investigated using the disc diffusion method, the agar cup method, and time–kill curves. We compared the antibacterial activities of the *Blumia lacera* Ag-NPs and *Neolamarckia cadamba* Ag-NPs against MRSA. The maximum antibacterial activity and highest survival rate of MRSA were observed in all undiluted solutions of *Blumia lacera* Ag-NPs. The viable count of the undiluted *Neolamarckia cadamba* Ag-NPs indicated that no survivors

survived after 2 hours of incubation, indicating that this agent is less effective at higher concentrations than are the *Blumia lacera* nanoparticles. The maximum zone of inhibition was observed for *Blumia lacera* compared to *Neolamarckia cadamba* nanosilver particles. Moreover, Ag-NPs promoted wound healing in Blumia lacera.

Recommendation

The present study clearly demonstrated the antibacterial activity and time-kill curves, which could potentially enhance wound healing. However, it is premature to comment on clinical cure in patients. Further studies are needed, including animal models and human volunteer trials, to assess the safety of this antimicrobial agent, particularly its cytotoxicity.

DECLARATION

Acknowledgement

Special thanks to the Vedantaa Institute of Medical Sciences, Vedantaa Hospital and Research Centre, Dahanu, for supporting this project.

Authorship Contributions

Concept and Writing: Dr. Harshada Shah and Kalpesh Khutade; Data Collection and Interpretation: Harshila Dhinde and Nisha Kumari.

Funding

The present research received no grant from any funding agency.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Vedantaa Instituteof Medical Sciences, Palghar (protocol code, EC/04/2023, date of approval 24 November 2023).

Consent for publication

Not applicable.

Competing interests

The authors declared that there is no conflict of interest.

REFERENCES

[1] Yang, D., Ding, M., Song, Y., Hu, Y., Xiu, W., Yuwen, L., Xie, Y. (2023): Nanotherapeutics with immunoregulatory functions for the treatment of bacterial infection. Biomaterials Research. 27(1):1-26. https://doi.org/10.1186/s40824-023-00405-7.

[2] Alsolami, A., Ghasab, A.L., Alharbi, M. S., Bashir, A. I., Saleem, M., Sye Khaja, A. S., Aldakheel, DF. (2023): Community-Acquired Methicillin-Resistant *Staphylococcus aureus* in Hospitals: Age-Specificity and Potential Zoonotic–Zooanthroponotic Transmission Dynamics. Diagnostics. 13(12):2089. https://doi.org/10.3390/diagnostics13122089.

[3] Aneja, A., Johnson, J., Prochaska, E. C., Milstone, A. M. (2024): Microbiome dysbiosis: a modifiable state and target to prevent *Staphylococcus aureus* infections and other diseases in neonates. Journal of Perinatology. 44(1):125-130. https://doi.org/10.1038/s41372-023-01810-5.

[4] Ahovan, Z. A., Esmaeili, Z., Eftekhari, B. S., Khosravimelal, S., Alehosseini, M., Orive, G., Dolatshahi-Pirouz, A. (2022): Antibacterial smart hydrogels: New hope for infectious wound management. Materials Today, Bio. 100499. https://doi.org/10.1016/j.mtbio.2022.100499.

[5] Rumata, N. R., Djide, N. J., Frediansyah, A., Nurul, A. M., Mutair, A., Alhumaid, S., Rabaan, A. A. (2023): Progress and Challenges in Antimicrobial Resistance and Bacterial Vaccines. Biointerface Research in Applied Chemistry. 13(5):1-21. https://doi.org/10.33263/BRIAC135.489.

[6] More, P. R., Pandit, S., Filippis, A. D., Franci, G., Mijakovic, I., Galdiero, M. (2023): Silver nanoparticles: bactericidal and mechanistic approach against drug resistant pathogens. Microorganisms.11(2):369. https://doi.org/10.3390/microorganisms11020369.

[7] Tamilarasi, G. P., Sabarees, G., Manikandan, K., Gouthaman, S., Alagarsamy, V., Solomon, V. R. (2023): Recent Trends in Electrospun Antibacterial Nanofibers for Chronic Wound Management. Current Nanomedicine (Formerly: Recent Patents on Nanomedicine). 13(3):159-187. http://dx.doi.org/10.2174/2468187313666230817151543.

[8] Menichetti, A., Mavridi-Printezi, A., Mordini, D., Montalti, M. (2023): Effect of Size, Shape and Surface Functionalization on the Antibacterial Activity of Silver Nanoparticles. Journal of Functional Biomaterials. 14(5):244. https://doi.org/10.3390/jfb14050244.

[9] Hosnedlova, B., Kabanov, D., Kepinska, M., Narayanan, V. H., Parikesit, A. A., Fernandez, C. B. (2022): Effect of Biosynthesized Silver Nanoparticles on Bacterial Biofilm Changes in *S. aureus* and *E. coli*. Nanomaterials. 12(13):2183. https://doi.org/10.3390/nano12132183.

[10] Li, H., You, Q., Feng, X., Zheng, C., Zeng, X., Xu, H. (2023): Effective treatment of *Staphylococcus aureus* infection with silver nanoparticles and silver ions. Journal of Drug Delivery Science and Technology. 80:104165. https://doi.org/10.1016/j.jddst.2023.104165.

[11] Singh, M., Thakur, V., Kumar, V., Raj, M., Gupta, S., Devi, N., Upadhyay, S. K. (2022): Silver nanoparticles and its mechanistic insight for chronic wound healing: review on recent progress. Molecules. 27(17):5587. https://doi.org/10.3390/molecules27175587.

[12] Lo, S., Mahmoudi, E., Fauzi, M. B. (2023): Applications of drug delivery systems, organic, and inorganic nanomaterials in wound healing. Discover Nano. 18(1):104. https://doi.org/10.1186/s11671-023-03880-y.

[13] Moaness, M., Mabrouk, M., Ahmed, M. M., Das, D. B., Beherei, H. H. (2022). Novel zinc-silver nanocages for drug delivery and wound healing: Preparation, characterization and antimicrobial activities. International Journal of Pharmaceutics. 616:121559. https://doi.org/10.1016/j.ijpharm.2022.121559.

[14] Chopra, H., Mohanta, Y. K., Mahanta, S., Mohanta, T. K., Singh, I., Avula, S. K., Mallick, SP. (2023). Recent updates in nanotechnological advances for wound healing: A narrative review. Nanotechnology Reviews. 12(1):20230129. https://doi.org/10.1515/ntrev-2023-0129.

[15] Khutade, K., Shah, H., Patil, S., Patel, H. (2023): Validation of an Assessment of Chromogenic Media Against Conventional Culture Techniques for Isolation, Identification, and Direct Antibiotic Susceptibility Testing of Uropathogens in Resource-Poor Settings. International Journal of Pharmacy and Biological Sciences (IJPBS).13(4):116–122. http://dx.doi.org/10.5281/zenodo.10516488.

[16] Asubel, F. M., Brent, R.., Kinston, R., Moore, D. E., Smith, J., Strunt, K. (1999): Protocols in Molecular Biology. 4th Edn, editor. New York: John wiley and sons Inc.

[17] Jonas, D., Speck, M., Daschner, F., Grundmann, H. (2002): Rapid PCR-Based Identification of Methicillin-Resistant Staphylococcus aureus from screening swabs. Journal Microbiology. 40:1821–1823. https://doi.org/10.1128/jcm.40.5.1821-1823.2002.

[18] Ninganagouda, S., Rathod, V., Jyoti, H., Singh, D., Prema, K., Haq, M. U. (2013): Extracellular biosynthesis of silver nanoparticles using Aspergillus flavus and their antimicrobial activity against gram-negative MDR strains. International Journal of Pharma and Bio Sciences. 4(2):222-229. www.ijpbs.net

[19] Audtarat, S., Hongsachart, P., Dasri, T., Chio-Srichan, S., Soontaranon, S., Wongsinlatam, W., Sompech ,S. (2022): Green synthesis of silver nanoparticles loaded into bacterial cellulose for antimicrobial application. Nanocomposites. 8(1):34-46. https://doi.org/10.1080/20550324.2022.2055375.

[20] Haque, M. A., Imamura, R., Brown, G. A., Krishnamurthi, V. R., Niyonshuti, II., Marcelle, T., Mathurin, L. E. (2017). An experiment-based model quantifying antimicrobial activity of silver nanoparticles on *Escherichia coli*. RSC advances. 7(89):56173-56182. https://doi.org/10.1039/C7RA10495B.

[21] Jena, P., Mohanty, S., Mallick, R., Jacob, B., Sonawane, A. (2012): Toxicity and antibacterial assessment of chitosancoated silver nanoparticles on human pathogens and macrophages. International Journal of Nanomedicine. 1805-1818. https://doi.org/10.2147/ijn.s28077.

[22] Gupta, K., Hazarika, S. N., Saikia, D., Namsa, N. D., Mandal, M. (2014): One step green synthesis and antimicrobial and anti-biofilm properties of Psidium guajava L. leaf extract-mediated silver nanoparticles. Materials Letters. 125:67-70. http://dx.doi.org/10.1016%2Fj.matlet.2014.03.134.

[23] Khutade, K., Chanda, S., Megha, G. K., Shah, H. (2022): Evaluation of Different Phenotypic Methods for Detection of Biofilm Formation among the Clinical Isolates. International Journal of Current Microbiology and Applied Sciences 11(10): 40-48. https://doi.org/10.20546/ijcmas.2022.1110.005.

[24] Bharathi, D., Vasantharaj, S., Bhuvaneshwari, V. (2018): Green synthesis of silver nanoparticles using Cordia dichotoma fruit extract and its enhanced antibacterial, anti-biofilm and photo catalytic activity. Materials Research Express. 5(5):055404. https://ui.adsabs.harvard.edu/link_gateway/2018MRE.....5e5404B/doi:10.1088/2053-1591/aac2ef.

[25] Singh, D., Rathod, V., Ninganagouda, S., Hiremath, J., Singh, A. K., Mathew, J. (2014): Optimization and characterization of silver nanoparticle by endophytic fungi Penicillium sp. isolated from Curcuma longa (turmeric) and application studies against MDR *E. coli* and *S. aureus*. Bioinorganic Chemistry and Applications.1-8.408021.https://doi.org/10.1155/2014/408021

[26] Bruna, T., Maldonado-Bravo, F., Jara, P., Caro, N. (2021): Silver Nanoparticles and Their Antibacterial Applications. International Journal of Molecular Sciences, *22*(13), 7202. https://doi.org/10.3390/ijms22137202.

[27] Bahrami, M., Shirazi, P. S., Moradi, F., Hadi, N., Navid, Sabbaghi., Sahba Eslaminezhad. (2024): How Nanomaterials Act against Bacterial Structures? A Narrative Review by Focusing on Molecular Mechanism of the Nanoparticles. Microbial Pathogenesis, 196, 107002–107002. https://doi.org/10.1016/j.micpath.2024.107002.

[28] Mondal, S., Chakraborty, S., Manna, S., Mandal, S. M. (2024): Antimicrobial Nanoparticles: Current Landscape and Future Challenges. RSC Pharmaceutics. 1, 388-402. https://doi.org/10.1039/D4PM00032C.

[29] Saha, Nakshi., Aklima, A., Sabonty, Bhattacharjee., Sabbir, Howlader., Abid, Hasan,. Harinarayan, Das. (2024): Phyto-extract-mediated Synthesis of Silver Nanoparticles using Blumea lacera Leaf Extract and Antimicrobial Activity Screening. Malaysian Journal of Chemistry. 26(2):96-109. https://doi.org/10.55373/mjchem.v26i2.96.

[30] Maheswari, J., Anjum, M. R., Sankari, M., Narasimha, G., Krishna, S. B. N., Kishori, B. (2023): Green synthesis, characterization and biological activities of silver nanoparticles synthesized from Neolamarkia cadamba. ADMET & DMPK. 11(4): 573–585. https://doi.org/10.5599/admet.1793.

[31] Haffner, S. M., & Malmsten, M. (2017). Membrane interactions and antimicrobial effects of inorganic nanoparticles. Advances in Colloid and Interface Science. *248*: 105-128. https://doi.org/10.1016/j.cis.2017.07.029.

[32] Menichetti, A., Mavridi-Printezi, A., Mordini, D., Montalti, M. (2023): Effect of size, shape and surface functionalization on the antibacterial activity of silver nanoparticles. Journal of Functional Biomaterials. 14(5):244. https://doi.org/10.3390/jfb14050244.

[33] Abbas, R., Luo, J., Qi, X., Naz, A., Khan, I. A., Liu, H., Wei, J. (2024): Silver nanoparticles: synthesis, structure, properties and applications. Nanomaterials. 14(17), 1425. https://doi.org/10.3390/nano14171425.

[34] Ameh, T., Zarzosa, K., Dickinson, J., Braswell, W. E., Sayes, C. M. (2023): Nanoparticle surface stabilizing agents influence antibacterial action. Frontiers in Microbiology. 14: 1119550. https://doi.org/10.3389/fmicb.2023.1119550.